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Title: Efficacy of 1% Hydrogen Peroxide Wash in Decontaminating Apples and Cantaloupe Melons

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Efficacy of 1% Hydrogen Peroxide Wash in Decontaminating Apples and Cantaloupe Melons

G.M. SAPERS AND J.E. SITES

ABSTRACT: Efficacy of 1% hydrogen peroxide (H_2O_2) in decontaminating apples and cantaloupes containing human pathogens was investigated. Apples inoculated with *Escherichia coli* (ATCC 25922) were washed with 1% H_2O_2 at 20 or 40 °C for 15 or 30 min. Population reductions approaching 3 logs were obtained with all treatments. Comparable reductions were obtained with apples inoculated with 3 strains of *E. coli* O157:H7, associated with cider outbreaks, and a 5-strain cocktail. The 1% H_2O_2 treatment was ineffective against *E. coli* 766 (ATCC 9637; similar to *Salmonella* Poona) on inoculated cantaloupes. Treatment of apples with 1% H_2O_2 was carried out successfully in a wet dump tank.

Keywords: apples, cantaloupes, decontamination, wash, *E. coli*, hydrogen peroxide

Introduction

IMPROVED METHODS OF DECONTAMINATING apples, melons, and other fruits and vegetables containing human pathogens are needed to reduce the risk of produce-related foodborne illness. Previous studies in our laboratory have demonstrated the efficacy of 5% hydrogen peroxide (H_2O_2), applied in conjunction with agitation, heating, vacuum infiltration, and abrasion, in inactivating a nonpathogenic *E. coli* on inoculated apples (Sapers and others 2002). Population reductions approaching 4 log₁₀ CFU/g were obtained with some treatments. In other studies, a 5% H_2O_2 wash, applied to the intact melon surface prior to cutting, was effective in extending the shelf-life of fresh-cut cantaloupe (Sapers and others 2001b) and in reducing the population of *E. coli* and *Salmonella* Stanley on the surface of inoculated cantaloupes (Ukuku and others 2001; Ukuku and Sapers 2001). Greater population reductions were obtained when the peroxide wash was applied to inoculated melons at 70 °C (Ukuku and others 2002). Although H_2O_2 is classified by the U.S. Food and Drug Administration as Generally Recognized as Safe (GRAS) for certain specified food applications (21CFR184.1366 2002), current FDA regulations do not permit use of H_2O_2 as a washing agent except when used at low concentrations in combination with acetic acid to form peroxyacetic acid and where the fruits and vegetables are not considered raw agricultural commodities (21CFR173.315 2002). However, a recent action by the U.S. Environmental Protection

Agency now exempts use of $\leq 1\%$ H_2O_2 , applied to all postharvest agricultural food commodities, from the requirement of a tolerance (40CFR180.1197 2002). A commercial H_2O_2 -based wash is being marketed for postharvest application to potatoes to control soft rot and other plant pathogens causing disease during storage; recommended use levels are between 0.27% and 0.54% (Biosafe Systems 2002). If a wash with $\leq 1\%$ H_2O_2 proved to be efficacious in inactivating human pathogens attached to produce, this treatment might be a viable alternative to use of chlorinated water or other sanitizer treatments.

In a previous study of mushroom-washing technology, we found that the microbial load in water used to wash dirty mushrooms could be reduced by about 2 logs if 0.5% H_2O_2 was used as the washing medium (Sapers and Simmons 1998). We developed a continuous mushroom-cleaning system in which a prewash with 0.5% or 1% H_2O_2 was followed by a 5% H_2O_2 wash. Mushrooms given the prewash showed fewer lesions, indicative of bacterial spoilage, than mushrooms cleaned without the prewash step. However, the 1% H_2O_2 prewash solution lost strength during prolonged use due to reaction with the mushrooms and suspended soil and had to be replaced periodically (Sapers and others 2001a). In other studies, we found that hot 1% H_2O_2 solutions reacted with metal surfaces in unpassivated stainless steel tanks, resulting in rapid loss of strength (Sapers and Sites 2001).

Fallik and others (1994) demonstrated

that treatment of eggplant and sweet red pepper with 0.5% Sanosil-25 (a proprietary disinfectant containing 0.24% H_2O_2 at this dilution) suppressed *Botrytis cinerea* and *Alternaria alternata* and reduced postharvest decay during storage. In a related study, Aharoni and others (1994) reported that the same concentration of Sanosil-25 reduced decay in Galia melons caused by *Alternaria* and *Fusarium*. Treatment with 1.0% Sanosil-25 was more effective, but induced browning of the peel. Crowe and others (2002) reported a reduction of 1 log or less in the microbial load on lowbush blueberries washed with 0.5% H_2O_2 . The combination of 1.5% lactic acid and 1.5% H_2O_2 , applied at 40 °C, was reported to be highly effective in inactivating *E. coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* on spot-inoculated apples, oranges, and tomatoes (Venkitanarayanan and others 2002). Treatment of lettuce with 2% H_2O_2 at 50 °C yielded population reductions of ≤ 4 logs for *E. coli* O157:H7 and *S. Enteritidis* and 3 logs for *L. monocytogenes*, with only slight browning during storage (Lin and others 2002). Lettuce given this treatment and stored at 5 °C for as long as 15 d was rated superior to or at least as high in quality as untreated controls by a consumer panel (McWatters and others 2002). However, it is not clear whether human pathogens attached to inaccessible sites, such as within the stem well and calyx of apples or in the stem scar and netting of cantaloupes, would have been inactivated by such treatments. Previous studies in our laboratory have dem-

onstrated survival of *E. coli* in such attachment sites during washing of dip-inoculated apples (Sapers and others 2000; Annous and others 2001).

Thus, there is a need to examine the efficacy of washing with $\leq 1\%$ H_2O_2 under conditions where bacterial inaccessibility may be a problem. Our objective in this study was to determine the efficacy of 1% H_2O_2 in inactivating *E. coli* in dip-inoculated apples and cantaloupes under conditions simulating a commercial produce wash treatment.

Materials and Methods

Source and inoculation of raw material

Unwaxed Washington State Golden Delicious apples weighing approximately 150 to 180 g were obtained from a single grower and stored at 4°C until needed for decontamination experiments (usually less than 2 months). Fresh cantaloupes (full slip), free of visual defects and weighing approximately 1.6 to 1.8 kg, were purchased at local food stores or from a distributor and stored at 4°C for no more than 1 wk prior to use. Apples and cantaloupes were not pre-washed by the investigators because they had been washed commercially prior to packing and were clean.

Apples were inoculated with a nonpathogenic *E. coli* strain, ATCC 25922, or a cocktail of *E. coli* O157:H7 strains SEA 13B88 (cider outbreak), Oklahoma (clinical isolate from 1999 Oklahoma cider-related outbreak), and C7927 (cider outbreak) or a cocktail of *E. coli* O157:H7 strains SEA 13B88, Oklahoma, C7927, F4546 (alfalfa seed), and WM 98A06026 (alfalfa seed). Inocula were prepared by growing the organism in trypticase soy broth (TSB; Difco, Detroit, Mich., U.S.A.) at 37°C for 8 h, transferring 100 μL of the late exponential-phase culture to 1 L of TSB, allowing the culture to grow for approximately 18 h at 37°C . Then the culture was centrifuged at $3874 \times g$ for 10 min, washed once with 200 mL sterile distilled water, and the cells were resuspended in 200 mL of sterile distilled water which, when diluted to 2 L with sterile distilled water, gave a final cell concentration of approximately $8.8 \log_{10}$ CFU/mL (range of 8.7 to $9.1 \log_{10}$ CFU/mL). For preparation of the cocktail, 40 mL portions of each individual undiluted *E. coli* O157:H7 culture were combined, and when diluted to 2 L with sterile distilled water, yielded an inoculum contain about $8.3 \log_{10}$ CFU/mL. Apples, taken from refrigerated storage, were immediately immersed in the stationary phase *E. coli* cell suspension at ambient temperature (about 20°C) for 5 min, drained, placed on their sides in plas-

tic tubs lined with absorbent paper, covered with aluminum foil, and held for 24 h at 4°C prior to use.

Individual cantaloupes were inoculated by immersion in a suspension of *E. coli* 766 (ATCC 9637), reported by Riordan and others (2002) to have similar attachment, growth, and survival characteristics as *Salmonella* Poona. This nonpathogenic *E. coli* was used instead of the human pathogen in anticipation of the need to conduct washing trials in an unprotected pilot plant dip or dump tank where *S. Poona* could not be used. The inoculum was prepared as described above for *E. coli* (ATCC 25922). Melons were inoculated by full immersion in 4 L of inoculum for 5 min, followed by draining on absorbent towels for 1 h on each side. Inoculated melons were placed in plastic tubs lined with absorbent paper, covered with aluminum foil, and stored at 4°C for 24 h.

Application of 1% H_2O_2 treatments

One hour prior to treatment, inoculated apples and melons were removed from refrigerated storage and equilibrated at room temperature. Sets of 3 inoculated apples were treated by immersion in 4 L 1% H_2O_2 in a 6.6-L cylindrical jar (Kimble nr 32600 618, Fisher Scientific, Pittsburgh, Pa., U.S.A.) that was covered with a stainless steel lid. The covered jar was placed in a shaker water bath (New Brunswick Scientific Innova Shaker, Model 3100, Fisher Scientific) operated at 120 rpm and 20°C (with chilled water supplied by a Neslab Refrigerating Circulator, Model RTE-211, Fisher Scientific) or at 40°C for 2, 15, or 30 min. Measured temperatures in the peroxide solutions at the beginning and end of apple treatments were within 1°C of the target temperatures. Following treatment, the apples were rinsed thoroughly under a strong stream of cool tap water (about 20°C) for 30 s. In trials with *E. coli* O157:H7, rinsing was by immersion for 30 s in 2 successive tubs containing 6 L deionized water; following rinsing, the water was decontaminated by addition of bleach prior to disposal.

These treatments were compared with 1% or 5% H_2O_2 at 60°C , applied for 2 min, and 200 mg/L Cl_2 (total chlorine, calculated from weight of added sodium hypochlorite; adjusted to pH 6.5 with HCl), applied at 20°C for 15 min. In 1 treatment, a wetting agent, 0.1% sodium 2-ethylhexyl sulfate (Stepanol SHS; Stepan Co., Northfield, Ill., U.S.A.) was added to 1% H_2O_2 to enhance penetration of peroxide into the calyx and stem areas.

Individual inoculated cantaloupes were treated with 1% H_2O_2 with the same appa-

ratus used for apples; however, the shaker was set to 100 rpm to avoid injury to the melons. Treatments were applied at 20°C for 15 min. In 1 experiment, 0.1% SHS (Stepan Co.) was added to the 1% H_2O_2 solution. Treated melons were rinsed with tap water for 30 s to remove H_2O_2 residues.

Application of 1% H_2O_2 treatments in a dump tank

Treatment of apples with 1% H_2O_2 was carried out in a 1000-L capacity stainless steel (304 series) dump tank, designed for produce processing research. The tank is provided with a temperature control system ($\pm 1^\circ\text{C}$) for operation at elevated temperatures and a process water circulation system that vigorously agitates the product in the water. Following treatment, the apples were transferred to a large plastic colander and rinsed with tap water using a hose.

Microbiological methods

Treated apples and inoculated controls (untreated apples from the same experiment, handled under the same conditions as the treated apples but not rinsed prior to blending) were weighed, cut into quarters on a sterile cutting board, combined with an equal volume (wt/vol) of sterile 0.1% peptone water (PW; Difco) and homogenized for 1 min at medium speed in a sterile 4-L stainless steel Waring blender (Waring Products Div., Dynamics Corp. of America, New Hartford, Conn., U.S.A.). Blended samples were filtered through a sterile filter bag designed for microbiological examination of particulate suspensions (40 μm pore size; Spiral Biotech, Bethesda, Md., U.S.A.), and the filtrates were diluted with sterile PW as required. Generic *E. coli* counts were estimated by spread plating 0.1 mL aliquots on trypticase soy agar (TSA; Difco), incubating for a minimum of 2 h at 37°C to allow injured cells to recover, and then overlaying with MacConkey agar (MAC; Difco). In trials with *E. coli* O157:H7, dilutions also were plated on Sorbital MacConkey agar (Difco) supplemented with cefixime (0.05 mg/L) and potassium tellurite 2.5 mg/L (CT-SMAC).

Rind plugs from treated cantaloupes and controls were prepared for microbiological evaluation as described (Sapers and others 2001). A composite sample of twenty plugs was taken at random locations on the surface of each melon with a sterile 20 mm dia stainless steel cork borer. The plugs were trimmed with a sterile knife to remove adhering flesh and then blended with 250 mL 0.1% PW in a 1250 mL glass jar with a single speed blender (Model 700; Waring Products Div., Dynamics Corp. of America). The *E. coli* 766 population size on the cantaloupe

Table 1—Comparison of 1% H₂O₂ with alternative wash treatments for inactivation of *E. coli* (ATCC 25922) in dip-inoculated Golden Delicious apples

| Treatment ^a | Time (min) | <i>E. coli</i> population reduction ^{b,c} (log ₁₀ CFU/g) |
|---|------------|--|
| 1% H ₂ O ₂ at 20 °C | 15 | 2.82 ± 0.46 |
| 1% H ₂ O ₂ at 20 °C | 30 | 2.94 ± 0.22 |
| 1% H ₂ O ₂ at 40 °C | 15 | 3.01 ± 0.27 |
| 1% H ₂ O ₂ at 40 °C | 30 | 3.15 ± 0.90 |
| 200 ppm Cl ₂ at 20 °C | 15 | 2.57 ± 0.35 |
| 5% H ₂ O ₂ at 60 °C | 2 | 3.51 ± 0.33 |
| 1% H ₂ O ₂ at 60 °C | 2 | 2.97 ± 0.68 |
| 1% H ₂ O ₂ at 20 °C | 2 | 2.19 ± 0.21 |

^aWith agitation provided by a shaker water bath at 120 rpm

^bMean of 3 to 4 replicate trials ± SD; reduction based on mean inoculated control population of 5.30 ± 0.08.

^cNo significant differences at *P* < 0.05 by Bonferroni LSD

surface was expressed as CFU/cm² rather than on a weight basis, calculated from the total count for 20 pooled plugs, divided by 20π (the rind surface area of 20 plugs, each having a radius of 1 cm).

Determination of H₂O₂ stability

Because of the possibility of H₂O₂ instability during treatment with the 1% solution, 200 μL aliquots of solution were taken periodically, diluted by addition to 200 mL deionized water, and analyzed for H₂O₂ with the Reflectoquant® Analysis System using Reflectoquant® Peroxide test strips (0.2 to 20 mg/L H₂O₂ range; EM Science, Gibbstown, N.J., U.S.A.). To determine the effect of apple solids (as might be leached from exposed flesh of mechanically damaged or decayed fruit) on the stability of 1% H₂O₂, the peroxide solution was “spiked” with fresh juice prepared from Golden Delicious apples without ascorbic acid addition, using an Acme Juicerator (Acme Juicer Mfg. Co., Lemoyne, Pa., U.S.A.) as described (Sapers 1991). The soluble solids content of the freshly obtained juice was determined with an Abbe-3L refractometer (Bausch & Lomb, Inc., Rochester, N.Y., U.S.A.) at 23 °C. The H₂O₂ concentration was determined in the freshly prepared 1% solution, immediately following addition of 0.1% or 0.2% juice, and then 15 and 30 min thereafter. In addition, Golden Delicious apples, previously inoculated with *E. coli* (ATCC 25922), were immersed in 1% H₂O₂ that had been spiked with 0.2% fresh apple juice to determine whether the presence of juice solids would interfere with treatment efficacy in reducing the *E. coli* population.

To confirm the stability of 1% H₂O₂ during treatments applied with vigorous agita-

Table 2—Effect of apple solids on efficacy of 1% H₂O₂ for inactivation of *E. coli* (ATCC 25922) in dip-inoculated Golden Delicious apples

| Treatment ^a | Added apple solids ^b | <i>E. coli</i> population reduction ^c (log ₁₀ CFU/g) | | H ₂ O ₂ (%) ^d |
|---|---------------------------------|--|------------|--|
| | | Before wash | After wash | |
| 1% H ₂ O ₂ at 20 °C | No | 2.70 ± 0.49 | 1.4 ± 0.08 | 1.4 ± 0.08 |
| 1% H ₂ O ₂ at 20 °C | Yes | 2.70 ± 0.42 | 1.2 ± 0.01 | 1.2 ± 0.02 |

^aFor 15 min with agitation provided by a shaker water bath at 120 rpm

^bAdded 0.2% fresh apple juice, equivalent to 0.026% apple soluble solids.

^cMean of 3 replicate trials ± SD; reduction based on mean inoculated control population of 5.18 ± 0.08; no significant differences at *P* < 0.05 by Bonferroni LSD.

^dDetermined by Reflectoquant® Analysis System

tion in the dump tank, aliquots of the solution were taken for analysis, as described above, initially and following 15 and 30 min trials conducted with 10 kg uninoculated apples at 40 °C. Peroxide concentrations during trials with inoculated apples also were monitored.

Statistical analyses

Population reduction data were analyzed for differences in response to treatments by analysis of variance and the Bonferroni least significant difference (LSD) test to separate means (Miller 1981). All statistical analyses were performed with SAS/STAT software (SAS Inst. Inc. 1989).

Results and Discussion

Efficacy of 1% H₂O₂ in decontaminating apples

Our initial experiments with apples indicated that treatment with 1% H₂O₂ at 20 or 40 °C over time spans of 10 to 30 min resulted in no discoloration with 20 °C treatments and slight darkening of lenticels at 40 or 60 °C. No losses of H₂O₂ strength occurred at either temperature under the conditions of these experiments. Treatment of apples, inoculated with a nonpathogenic *E. coli* (ATCC 25922), with 1% H₂O₂ for 15 or 30 min at 20 or 40 °C yielded population reductions approaching 3 logs, with treatment time and temperature having little or no effect (Table 1). Addition of a wetting agent (0.1% SHS) had no effect on the log reduction obtained with 1% H₂O₂ applied at 20 °C for 15 min (data not shown). In side-by-side comparisons, both 1% H₂O₂ treatments at 20 and 40 °C were similar in efficacy to 200 mg/L Cl₂ at 20 °C, applied for 15 min, and to 1 or 5% H₂O₂, applied at 60 °C for 2 min. Thus, low concentrations of H₂O₂, applied at ambient or slightly elevated temperatures for 15 and 30 min, are at least as effective as Cl₂ as a sanitizer for apples. These treatments could not be applied in a brush washer or dip tank, which generally employ residence times of about 1 min or less, but might be

applied in a wet dump tank where the required times and temperatures would be compatible with fruit packing operations.

Spiking 1% H₂O₂ with as much as 0.2% fresh apple juice (equivalent to an added soluble solids content of 0.026%) had no effect on H₂O₂ stability during 15 or 30 min at ambient temperature (23 °C). The efficacy of 1% H₂O₂ in reducing the *E. coli* population on inoculated apples was unimpaired by addition of 0.2% juice to the peroxide solution (Table 2). These results indicate that the presence of soluble solids leached from fragments of decayed or mechanically damaged apples would not be likely to affect the efficacy of the 1% H₂O₂ treatment.

To confirm the results of washing trials with the surrogate, apples were inoculated with individual strains or a 5-strain cocktail of *E. coli* O157:H7. Population reductions obtained with 1% H₂O₂ were similar at 20 and 40 °C and also comparable to reductions obtained with 5% H₂O₂ applied at 60 °C for 2 min (Table 3). Population reductions with the latter treatment and with 1% H₂O₂ at 40 °C were higher than reductions obtained with 200 mg/L Cl₂ (CT-SMAC data). Examination of the pooled treatment data indicated that population reductions were greater with the Oklahoma strain and the cocktail than with the SEA 13B88 or C7927 strains. Population reductions with the Oklahoma strain of *E. coli* O157:H7 and 5-strain cocktail were comparable to those obtained with the surrogate (Table 1).

It is likely that the size of the *E. coli* O157:H7 population surviving treatment was determined as much by the inaccessibility of attachment sites on calyx and stem surfaces as by strain-to-strain differences in strength of attachment or resistance to H₂O₂. In previous washing studies with various sanitizers and surfactants, we have demonstrated survival of *E. coli* in the stem and calyx areas of inoculated apples (Sapers and others 1999, 2000, 2002; Riordan and others 2002). Recovery of *E. coli* O157:H7 populations from inoculated apples on TSA/MAC and CT-SMAC was not signifi-

Table 3—Response of *E. coli* O157:H7 strains on dip-inoculated Golden Delicious apples to 1% H₂O₂ and alternative wash treatments

| Strain | Media ^b | <i>E. coli</i> O157:H7 population reduction (log ₁₀ CFU/g) ^a | | | | |
|------------------------------|--------------------|--|---|----------------------------------|--|-----------------------------|
| | | 1% H ₂ O ₂ at 20 °C | 1% H ₂ O ₂ at 40 °C | 200 ppm Cl ₂ at 20 °C | 5% H ₂ O ₂ at 60 °C ^c | All treatments ^d |
| SEA 13B88 | TSA/MAC | 2.16 ± 0.01 | 2.40 ± 0.43 | 1.87 ± 0.08 | 2.10 ± 0.30 | 2.06 g |
| | CT-SMAC | 2.12 ± 0.23 | 2.11 ± 0.27 | 1.23 ± 0.19 | 2.47 ± 0.69 | |
| C7927 | TSA/MAC | 1.94 ± 0.16 | 1.94 ± 0.47 | 1.70 ± 0.09 | 2.58 ± 0.40 | 1.97 g |
| | CT-SMAC | 1.78 ± 0.13 | 2.16 ± 0.88 | 1.60 ± 0.15 | 2.38 ± 0.82 | |
| Oklahoma | TSA/MAC | 2.58 ± 0.22 | 2.31 ± 0.29 | 2.29 ± 0.13 | 2.80 ± 0.40 | 2.60 f |
| | CT-SMAC | 2.87 ± 0.80 | 2.71 ± 0.28 | 2.09 ± 0.07 | 3.06 ± 0.31 | |
| Cocktail ^e | TSA/MAC | 2.49 ± 0.13 | 2.86 ± 0.16 | 2.51 ± 0.06 | 2.93 ± 0.13 | 2.89 f |
| | CT-SMAC | 2.54 ± 0.22 | 3.52 ± 0.42 | 2.32 ± 0.12 | 3.92 ± 0.78 | |
| 3 cider strains and cocktail | TSA/MAC | 2.29 Ff | 2.38 Ff | 2.09 Ff | 2.52 Ff | — |
| | CT-SMAC | 2.34 FGf | 2.63 Ff | 1.81 Gf | 2.95 Ff | |

^aMeans of duplicate trials ± SD; population reductions based on mean inoculated control populations for each strain or the cocktail (4.43 to 4.98 log₁₀CFU/g on CT-SMAC and 4.85 to 5.67 log₁₀CFU/g on TSA/MAC).

^bTSA overlaid with MAC after 2 h for recovery or CT-SMAC.

^cTreatment with 5% H₂O₂ at 60 °C applied for 2 min; all other treatments applied for 15 min.

^dData for both media pooled

^eFive strain cocktail comprising SEA 13B88, Oklahoma, C7927, F4546, and WM 98A06026.

^fWithin the same column (small letters) or row (capital letters), means with no letter in common are significantly different ($P < 0.05$) by Bonferroni LSD.

Table 4—Population reduction in cantaloupes dip inoculated with *E. coli* (ATCC 9637) and treated with 1% H₂O₂

| Treatment ^a | <i>E. coli</i> population reduction ^b (log ₁₀ CFU/g) |
|---|--|
| 1% H ₂ O ₂ at 20 °C | 1.10 ± 0.61 |
| 1% H ₂ O ₂ containing 0.1% SHS at 20 °C | 0.51 ± 0.17 |

^aEach melon was immersed in treatment solution for 15 min.

^bMean ± SD; reductions based on mean of 3 trials with individual melons for each treatment and 3 corresponding inoculated control melons; no significant differences at $P < 0.05$ by Bonferroni LSD.

Table 5—Efficacy of 1% H₂O₂ wash treatments, applied in a dump tank, for inactivation of *E. coli* (ATCC 25922) in dip-inoculated Golden Delicious apples

| Treatment ^a | Time (min) | <i>E. coli</i> population reduction ^b (log ₁₀ CFU/g) | | H ₂ O ₂ concentration (%) ^c |
|---|---------------|--|------------|---|
| | | | | |
| 1% H ₂ O ₂ at 20 °C | 15 | 2.71 ± 0.38 | 1.2 to 1.3 | |
| | 30 | 2.84 ± 0.38 | 1.4 to 1.6 | |
| 1% H ₂ O ₂ at 40 °C | 15 | 2.73 ± 0.57 | 1.2 to 1.4 | |
| | 30 | 2.69 ± 0.50 | 1.2 to 1.3 | |

^aApples were treated by immersion in 1% H₂O₂ solution in dump tank with vigorous agitation sufficient to wet all surfaces.

^bMeans of 4 replicate trials ± SD; reduction based on mean inoculated control population of 4.92 ± 0.09; no significant differences at $P < 0.05$ by Bonferroni LSD.

^cDetermined by Reflectoquant® Analysis System

cantly different (Table 3). The similarity of population reductions determined with the 2 media for corresponding treatments suggests that treatment-induced injury was not an important factor in determining survival to these treatments. Thus, washing apples naturally contaminated with *E. coli* O157:H7 using 1% H₂O₂ at ambient temperature might be expected to reduce the pathogen load by more than 2 logs (average of 2.3 at 20 °C and 2.5 logs at 40 °C for the 2 media).

No skin discoloration was observed in apples treated with 1% H₂O₂ at 20 or 40 °C. Use of low concentrations of H₂O₂ at near ambient temperature avoids the possibility of lenticel darkening and skin browning, as observed previously with 5% H₂O₂ or hot water treatments applied to apples at temperatures > 60 °C (Sapers and others 2002).

Efficacy of 1% H₂O₂ in decontaminating cantaloupes

In contrast to the apple results, treatment of inoculated cantaloupes with 1% H₂O₂ was relatively ineffective in reducing the population of *E. coli* 766 (Table 4). Population

reductions were ≤ 1 log, and melon-to-melon variability in treatment response was high. Population reductions were not affected by the addition of SHS, a wetting agent, to the 1% H₂O₂ solution. In previous studies with cantaloupes inoculated with a nonpathogenic *E. coli*, population reductions exceeded 2 logs with 5% H₂O₂ treatment applied at ambient temperature (Ukuku and others 2001). In similar studies with *S. Stanley* as the test organism, population reductions were ≤ 2 logs when the inoculated melons were held for 24 h prior to treatment as was done in the present study (Ukuku and Sapers 2001).

Immersion of cantaloupes in 1% H₂O₂ for as long as 2 h had little or no effect on the bulk peroxide concentration in the solution (data not shown). It is possible that peroxide depletion occurred in localized areas within or on the netting and in other surface features where bacteria might attach. Such depletion might result from oxidation reactions at the melon surface or from access to endogenous catalase.

No further work was done on cantaloupes in this study in view of the minimal

efficacy of the 1% H₂O₂ treatment and the availability of other options, such as immersion in hot water or hot 5% H₂O₂ solution at 70 to 80 °C, that yield substantially greater population reductions (Ukuku and others 2002).

Application of 1% H₂O₂ wash treatments in a dump tank

A preliminary trial with uninoculated apples revealed little or no loss in peroxide strength over 30 min at 40 °C. Bubble formation on tank stainless steel surfaces and welds, an indication of H₂O₂ breakdown to O₂ and water, also was minimal. Rapid loss of strength accompanied by vigorous bubbling had been observed previously in a cantaloupe experiment with 1% H₂O₂ at 70 °C, carried out in a stainless steel dip tank (Ukuku and others 2002). Application of the 1% H₂O₂ treatment to inoculated apples in the dump tank yielded population reductions comparable to those obtained in laboratory-scale experiments with no loss in peroxide strength over several hours of operation (Table 5). Such reductions do not approach the 5-log reduction mandated for apple ci-

der and other fresh juices (FDA 2001), but could have a significant impact on the risk of exposure to *E. coli* O157:H7 or other human pathogens from fresh market or fresh-cut apples. With the latter, survival of human pathogens in the stem and calyx areas during washing of contaminated raw material could result in transfer to the fresh-cut slices during coring and slicing operations, and subsequent growth on cut surfaces, as demonstrated by Janisiewicz and others (1999), Conway and others (2000), and Gunes and Hotchkiss (2002) for *E. coli* and *L. monocytogenes*. Our results indicate that a 1% H₂O₂ treatment shows promise as an alternative to use of Cl₂. This treatment has no adverse effect on apple appearance and leaves no H₂O₂ residue. Whether the peroxide treatment would be considered commercially feasible by apple packers and fresh-cut processors will depend on regulatory decisions and the compatibility of the treatment with commercial raw material handling practices and equipment.

Conclusion

POPULATION REDUCTIONS OBTAINED IN apples inoculated with *E. coli* O157:H7 and washed with 1% H₂O₂, as described herein, are comparable to reductions obtained by treatment with 5% H₂O₂ under high-temperature, short-time conditions, and at least as large as those obtained with 200 ppm chlorine. The 1% H₂O₂ treatment was less effective in decontaminating inoculated cantaloupes. The 1% H₂O₂ apple treatments can be applied in a wet dump tank.

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